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WITNESS my hand this
Second day of July 2003

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Regulation 3.2

VISION BIOSYSTEMS LIMITED

A U S T R A L I A

Patents Act 1990

PROVISIONAL SPECIFICATION

for the invention entitled:

"A METHOD AND APPARATUS FOR PROVIDING A REACTION CHAMBER"

The invention is described in the following statement:

A METHOD AND APPARATUS FOR PROVIDING A REACTION CHAMBER

Field of the Invention

The present invention relates to a method or apparatus for providing a reaction chamber for chemical reactions. The present invention also relates to a method of filling a reaction chamber and a fluid used for this purpose.

Background of the invention

There are many applications where it is desirable to initiate a chemical reaction on a sample. Commonly the samples are located on a microscope slide. Typical reactions include immuno-histochemical reactions of cellular material, or in situ-hybridisation of DNA or RNA. In other forms, microarrays of thousands of small samples of material, including DNA, RNA proteins or small chemical compounds are attached to a microscope slide, where it is desirable to promote a chemical reaction between the material on the slide and other chemicals or fluids. These reactions require controlled conditions, including controlled reaction time, temperature and concentration of chemicals. It is important that the reaction across the slide is uniform, and also that reactions from slide to slide are consistent.

It is also important to minimise evaporation and overall fluid quantity used.

In the past, chemical reactions taking place on slides have been controlled by skilled persons adding and mixing the reactants. This allowed the time and quantity of the reactants to be controlled for each slide. However, this procedure is time consuming, required highly skilled operators, and can produce inconsistent results from slide to slide.

Summary of the Invention

In one form, the present invention is a biological reaction apparatus for receiving at least one substrate having a sample located in a sample region, and a separate covertile, such that a reaction chamber is formed between the covertile and substrate over the sample region, wherein the apparatus includes

a locating means to locate the substrate;

a covertile locating means for locating and moving the covertile with respect to the substrate;

a fluid dispensing means for dispensing fluid into the reaction chamber; and

a draining mechanism;

wherein the draining mechanism includes wicking means.

More preferably the wicking means include points of contact on the substrate to provide a fluid path to drain fluid from the slide.

Preferably the substrates are supported in the apparatus from underneath. Supporting substrates from underneath removes wicking paths from a round the periphery of the substrate, which reduces fluid usage and loss.

In another form, the present invention provides a fill fluid for performing a filling of a reaction chamber, where the fill fluid has a viscosity higher than an antecedent fluid on the substrate.

Preferably the fill fluid is miscible with water

Preferably the fill fluid has a higher boiling point than water.

Preferably the fill fluid leaves no residue on the substrate or sample.

Preferably the fill fluid is inert to biological reagents and samples.

Preferably the fill fluid is a solution comprising glycerol.

In one form the fill fluid contains glycerol, water, and buffer. The buffer may be tris buffered saline.

Preferably the fill fluid contains between 2% to 80% glycerol by volume.

More preferably still the fill fluid contains between 10%-60% glycerol per volume.

More preferably the fill fluid contains between 20% to 30% glycerol.

In one form the fill fluid includes a surfactant to aid in the disbursement of any bubbles formed within the reaction chamber during a fill cycle.

More preferably the surfactant is Tween.

In another form the present invention relates to a receptacle for substrates having receiving means adapted to locate a substrate and a covertile.

Preferably the receiving stations locate and support the substrate, and the covertile is supported on the substrate.

Preferably the receiving stations support the substrate around part of a periphery of the substrate.

Preferably the receiving means are defined by a respective aperture having peripheral ledges for supporting the slides.

Preferably the apertures are adapted to receive support platforms from a reaction apparatus, such that when loaded in a reaction apparatus, the platforms support the substrates.

Preferably the receiving means have a lifting means for lifting the covertiles from the substrate.

More preferably the lifting means are ramps adapted to engage with projections on the covertile.

Preferably the receiving means have guides allowing the covertile to be moved with respect to the receptacle and slide.

In another form the present invention relates to a dispenser for a reaction apparatus including a fluid conduit,

a pump connected to the fluid conduit;

a locating means for moving the fluid conduit from a fluid source to a dispensing region.

Preferably the dispenser includes a bar code sensor to detect the type of fluid source and substrate;

Preferably the dispenser includes a means for determining the volume of fluid remaining in a fluid source.

More preferably the means for determining the volume of fluid in a fluid source includes a sensor adapted to measure the level of fluid in a fluid container.

More preferably the sensor measures a change of capacitance of the fluid conduit to detect insertion into a fluid in the fluid container.

In another form the present invention relates to a method of dispensing fluid to a slide including the steps of:

Loading a reagent receptacle with at least one fluid container;

Mounting the reagent receptacle to a reaction apparatus

Detecting the reagent receptacle

Once the reagent receptacle in detected, initiating a sensor to detect the type of fluid within the at least one fluid container

Storing the information on fluid type to allow the fluid to be dispensed onto a slide when required.

Preferably the sensor detects bar codes.

In another form the present invention relates to a reaction apparatus having a support projection for a slide, a dispensing means and a fluid removal means, where the a support projection is adapted to support a slide from underneath, and a wicking means contacting the periphery of the slide, such that the wicking means provides a wicking path to remove fluid from the upper surface of the slide.

Preferably the a support projection is angled between 0 and 10 degrees to the horizontal providing the mount with a fluid removal region. This provides a gradient to promote fluid flow.

Preferably the wicking means is wicking posts.

Preferably the wicking posts are located at the fluid removal region.

In one form the wicking means is adapted to extend across a significant proportion of the width of the slide.

In another form the present invention relates to a reaction apparatus adapted to locate a substrate having a surface containing a sample and covertile having a surface forming a reaction chamber with the sample containing surface, including a covertile engaging means adapted to change the volume of the reaction chamber.

This promotes mixing of fluid within the reaction chamber.

In one form the covertile engaging means is a clamping mechanism adapted to clamp the covertile to the substrate.

In another form the present invention relates to a reaction apparatus having a separate substrate tray:

the substrate tray adapted to hold number of slides and covertiles;

At least one receiving station for receiving said substrate tray;

A dispensing means for dispensing fluid onto substrates in the substrate tray

Wherein a reaction chamber is formed between the slide and covertile, such that fluid dispensed onto the slides enters the reaction chamber.

Preferably the reaction apparatus has a number of receiving stations, each station adapted to receive a substrate tray.

Preferably the reaction apparatus has a controller which allows the fluid to be dispensed onto a substrate on one substrate tray independently of any other substrate tray.

In another aspect, there is provided reaction apparatus for receiving a substrate having a sample located in a sample region and a draining mechanism including wicking means for draining fluid from the substrate.

Specific examples of the abovementioned inventions will be discussed, with reference to the following figures.

Brief Description of the Drawings

Figure 1 shows an example of a reaction apparatus;

Figure 2 shows an example of a tray used with the reaction apparatus of figure 1;

Figure 3 shows the tray of figure 2 partially loaded into a receiving port of the reaction apparatus of figure1;

Figure 4 shows an example of a reagent container rack and rack receiving zone of the reaction apparatus;

Figure 5 shows the robotic arm and dispensing mechanism of the reaction apparatus of figure 1;

Figure 6 shows slides and covertiles loaded onto stations of a reaction apparatus of figure 1;

Figure 7 shows a covertile loaded into a tray shown in figure 2;

Figure 8(a) – (c) shows a covertile in three positions relative to a slide;

Figure 9 shows a first view of an engaging means for a covertile in a receiving port of the reaction apparatus of figure 1;

Figures 10 shows a schematic section of a reaction chamber formed between a covertile and a slide;

Figure 11 shows a washing station for the reaction apparatus;

Figure 12 shows a station of a tray receiving port and wicking means.

Figure 13 shows a cut away section of a covertile mounted upon a slide;

Figure 14 shows a top view of tray receiving port of the reaction apparatus of figure 1;

Figure 15 shows a cross section of a slide and covertile on a mount of a station;

Figure 16 shows a cutaway sections of the slide, covertile, and mount of figure 15.

Figure 1 shows an automated reaction apparatus 10 having bulk reagent container receiving zone 12, substrate tray receiving ports 14, a robotic arm 16 and a reagent rack receiving zone 18.

Bulk container receiving zone 12 is adapted to hold a number of bulk reagent containers 20. These containers 20 typically hold fluids such as tris buffered saline,

PBS, Citrate, EDTA, organic solvents, waste reagents, deionised water, and dewaxing solutions. The containers of the apparatus 10 hold 1 to 4 litres of fluid.

The robotic arm 16 is moveable along the guide 24, driven by motors (not shown) and controlled by a controller (not shown) such as a computer. As shown in figure 5 a dispensing means 26 is moveably mounted to arm 16, and includes a fluid conduit such as pipette 28, for dispensing fluids. The pipette 28 is attached by tubing 29 to a pump (not shown) which in this example is a motorised syringe pump capable of withdrawing, holding and delivering an accurate volume of fluid. The pipette 28 may be lowered when withdrawing or dispensing fluids, and raised when moving across the apparatus 10. A sensor 33 for reading bar codes is also included on the arm 16.

The reagent rack receiving zone 18 includes 4 rack mounts 30, rack locating clip 31 and a sensor 35 for detecting the mounting of each reagent rack 34, as best seen in figure 4. The reagent racks 34 each includes ten receptacles 36, each adapted to receive a reagent container 39. The reagent racks 34 may be removed from the rack receiving zone 18 when it is necessary to remove, refill or change a container 39.

In figures 1 and 3 there are three slide tray receiving ports 14 and each is adapted to hold a single slide tray 15.

The slide tray 15 (shown in figure 2) includes ten slide receiving means 37, in the form of apertures which have support means 38. One or more substrates in the form of slides 1 may be placed into the slide tray 15, as shown in figure 3, such that the slides 1 are supported around the periphery but not in the middle. Covertiles 2 are placed onto the slides 1 as shown in figure 7. When the slide tray 15 is placed into the tray receiving port 14, each receiving means 37 corresponds to a slide station 35 in the apparatus 10 as shown in figure 6 and described in further detail below. A series of blocks 40 in the tray receiving ports 14 are adapted to support the slides 1 when the slide tray 15 is fully inserted into the apparatus 10 along rails 39. When the slide tray 15 is inserted fully into the receiving port 14, it may be lowered such that the slides come into contact with and are supported by the blocks 40. The slide tray 15 is then not in contact with the slides, leaving the slides supported from underneath by the blocks 40. While only two slides 1 and covertiles 2 are shown loaded onto the tray 15

shown in figure 3, there may be any number of slides and covertiles, up to the number of receiving means 37 contained by slide tray 15.

The blocks 40, which are typically metal and may be controllably heated or cooled, support the slides 1 in conjunction with wicking means 41 in the form of wicking posts 42 as shown in figure 12. The upper surface of blocks 40 are inclined at a small angle to the horizontal (typically 5 degrees) to promote fluid flow along the slide during operation of the apparatus 10.

The covertile 2 (best seen in figures 8 and 13) is one of a number of variations possible, other variations being described in copending application titled "A covertile for a slide" by the same applicant and hereby incorporated by reference. The covertile 2 is made from a clear plastic material, and is substantially the same width as the slide 1 to which it is to be mounted. A recess 51 is located on side of the covertile 2 that faces the sample, and this recess 51 in conjunction lands 52 and sample holding surface 53 of the slide forms a reaction chamber 32 as shown in schematic figure 10 , where the z axis has been exaggerated for clarity. Figure 10 is a sectioned view of a covertile over a slide 1 showing the reaction chamber 32, sample 5, lands 52 and slide surface 53. Typically the slide is 25mm wide by 76mm long, and the recess is 100 micrometres high. The land 52 is in close proximity to or contacts slide surface 53 along contact surface 54 as shown in figure 13, and therefore restricts fluid leakage from the reaction chamber 32 outside the reaction chamber. Capillary forces assist in holding the fluid in the reaction chamber 32.

A locator arm 3 enables the covertile 2 to be moved along the slide 1 by a locator engaging means 43 shown in figure 9. Each locator arm 3 is engaged by a bracket 44. A range of positions of the covertile relative to the slides is shown in figure 8, where figure 8 (a) is fully open, figure 8(b) is partially open and figure 8(c) is fully closed. A reaction chamber 32 is formed between the covertile 2 and slide 1 over a sample 5 on the slide 1 when the covertile is in a closed or partially open position. The covertile 2 includes a fluid reservoir 19 where fluid may be dispensed. There are several forms of fluid reservoir, as described in the abovementioned copending

application. The covertile and slide are capable of holding fluid in the reservoir 19. when the covertile is in contact with the slide.

The fluid in the reservoir is drawn into the recess 51 of the covertile as the covertile moves over the slide from an open position shown in figure 8(a) to a closed position shown in figure 8(c). The reservoir 19 may hold sufficient volume such that there is still fluid in the reservoir when the covertile is in a closed position, and this provides a reservoir of fluid to reduce the need for fluid top ups during extended reaction times or sustained high temperatures. It is believed that the fluid is drawn into the recess by a number of factors including capillary forces.

The covertiles 2 include wings 50 projecting from covertile 2 adapted to engage ramps 52 on the slide tray 15, as shown in figure 7. The wings lift the covertile 2 clear from the slide 1 when the wings 50 on the covertile 2 engage lifting means in the form of ramps 52. It is possible to move the covertile 2 to a position where the sample is uncovered but the covertile remains in contact with the slide, along guides 56. Depending on the configuration of the ramps 52 and wings 50, it may not be necessary to completely open the chamber before the covertile loses contact with the slide 1.

The arm 3 is moved by an actuator such as a cam arrangement (not shown) which engages positioning member 45 controllably so that the covertile is able to be accurately positioned with respect to the slide along the x-axis shown in figure 8. While figure 9 shows that all covertiles are moved at once, in other examples of reaction apparatus it is possible to have individual control of the covertiles by moving arms individually.

In figure 6, slides 1 having bar codes 6 are shown on their respective blocks 40. For the purposes of this diagram the slide tray 15 and engaging means 43 have been omitted from view for clarity. A clamp 60 is used to hold a covertile 2 securely in position on the slide1during a processing step. Clamp 60 includes a number of legs 62, which are situated around the periphery of the slide 1 and have spring like properties to provide an even force around the periphery of the covertile. The clamp 60 may be made from a plastic material, and in another example (not shown) the legs

may be made from metal, in the form of a spring (leaf or coil). Other forms of legs or clamp are possible such as compressible foam or pneumatic clamps.

The clamp 60 for each covertile 2 may be raised when the covertile 2 is to be moved, or lowered to engage the covertile 2 during a fluid dispensing operation. In the present example, all clamps 60 and covertiles 2 in a particular receiving port 14 are moved together. Individual receiving ports 14 may operate independently of each other.

In use, bulk reagents in bulk reagent containers 20 are loaded into the apparatus 10. Reagent racks 34 having reagent containers 39 are loaded into the rack mounts 30. Sensors 35 detect their presence and the bar code sensor 33 reads the bar codes on each reagent container 38 to identify the contents of each reagent container relative to position in the reagent rack. Information relating bar codes 6 on slides 1 to samples on the slides and bar codes on reagent containers relating to contents, is input into the controller (not shown), which is typically a computer work station having an appropriate software interface and drivers. A slide tray 15 containing at least one slide 1, but up to ten slides, is placed into the receiving port 14, whereupon a sensor (not shown) detects the slide tray 15 and initiates a scan of the stations 35. When scanning, the bar code sensor 33 on the robotic arm 16 moves to each station 35 and attempts to read a bar code. If a slide with a bar code is present, the controller compares the bar code with a list of known slides and information input by the user to determine which protocol to apply to each individual slide. Alternatively, once the bar codes have been scanned, the user inputs information required for the apparatus to process the slide. Each slide may have a different protocol. The controller compares the reagents required to perform the reactions dictated by the protocols with the reagents located in the containers in the reagent racks 34. Any discrepancy will cause an error message to be sent to the user. If a reagent container 39 is missing then the reagent rack 34 may be removed and the correct container 39 placed in the rack 34, whereupon the rack is detected and a another scan of reagent containers is undertaken.

If no errors are present, the robotic arm 16 moves the pipette 28 of the dispensing means 26 moves to the appropriate reagent container 38 and withdraws the required

amount of fluid. At this time the dispensing means 26 checks the capacitance of the pipette 28, which changes when the pipette comes into contact with the fluid surface of the reagent container 39. In this way the volume of fluid remaining in the reagent container can be determined and the user can replace the container as necessary. The robotic arm then moves the pipette 28 to the first slide (determined by the controller) and dispenses the fluid onto the surface of the slide. There are several options in placement of the pipette and covertile in relation to the sample on the slide, and these will be discussed further below.

Once the dispensing operation for a first slide has been undertaken, the process is repeated for further slides. It is not necessary for each slide to be filled with the same fluid at each step, and the slides may be filled in any order that is appropriate. A washing station 120 shown in figure 11 is located near the reagent racks 34 may be used to clean the pipette 28 prior to withdrawal of a different reagent. Washing station 120 includes a receptacle 121 for receiving the pipette 28, where cleaning fluid from one of the bulk reagent c ontainers is pumped onto the outside of the pipette to remove traces of the previous fluid. Cleaning fluid may also be pumped from the bulk reagent container via tubing to clean the inside surfaces of the pippette.

Also located adjacent the washing station is a mixing station 122 shown in figure 11. Mixing vessels (not shown) may be placed into receivers 123 to provide a vessel to temporarily hold fluid. The pipette 28 may draw fluid from a container and place it into a mixing vessel, obtain fluid from one or more different containers and place it into the mixing vessel, whereby the combined fluids may then by drawn back into the pipette and dispensed onto a slide. The mixing vessels may be of different size depending on the quantity of fluid required. A mixing vessel may have sufficient volume to supply several reaction chambers with sufficient volume.

The mixing station provides the benefit of enabling fluids with short shelf lives to mixed only when required. This mixing may also be accomplished automatically without operator assistance. This reduces the chance of error.

Reagents may be pumped from the bulk reagent containers through piping and valves (not shown) into the pipette 28. Bulk reagent from the bulk reagent containers may also by pumped to a wash station.

When a slide tray 15 is loaded into the apparatus, each arm 44 is engaging the locator 3 of each covertile 2 in the slide tray 15. If an open fill is required, ie where the covertile 2 is substantially or fully withdrawn from the slide 1, the arm 44 moves all covertiles 2 on the slide tray 15 off the slides to a position such as that shown by covertile 2 in figure 8(a). This open position of the covertile 2 exposes the sample 5, whereupon the pipette 28 may be positioned in a variety of positions. The positions of the pipette 28 include either over the sample 5, to dispense fluid directly onto the pipette 28, or adjacent the front of the covertile 2 into a fluid reservoir 19 shown in figure 8. The reasons for each position will be explained below.

In an open fill situation, once the fluid has been dispensed on all slides, the arm 43 moves to position the reaction chambers over the samples on the slides. Capillary action and the movement of the covertile 2 over the surface of the slide 1 causes dispensed fluid to flow into the region between the covertile 2 and slide 1. The clamp 60 may be used to hold the covertile 2 in place and prevent it from floating on the film of liquid between the covertile and slide.

When the slide 1 is on the block 40, it may be in contact with wicking posts 42, as shown in figures 14 and 15. Movement of the slide 1 on the block 40 is possible as slide lengths vary, and movement of the covertile 2 over the slide can move the slide 1. Normally this movement is only in the order of 1-2mm. In another example (not shown) it is possible to use an actuator to move the slide away from the wicking posts to reduce wicking of fluid from the reaction chamber.

Figure 15 shows the covertile 2 on the slide 1, both located on block 40. The wicking posts 42 are in contact with the slide and therefore provide a wicking path for fluid. The reaction chamber is located between the slide and covertile but as figure 15 is approximately to scale, it cannot be clearly seen in this view. Fluid entered in fluid reservoir 19 flows into the reaction chamber and may flow from the reaction chamber down drain 55 associated with the wicking posts 42. To assist in fluid clearance, the

air pressure around the wicking posts may be lowered by withdrawing air from the drain 55 by a pump such as a fan (not shown). This will promote fluid flow through the reaction chamber and out the drain 55 if required. Withdrawing the covertile from the slide will also promote fluid flow down the drain 55.

The wicking posts will wick fluid even if not touching the slide, as the meniscus of the fluid will extend out from the edge of the slide near the wicking posts if there is fluid pressure from the wicking posts, or if the air pressure in that region is reduced.

When dispensed fluid fills the reaction chamber 32 there may be fluid contact between the fluid in the reaction chamber and the wicking posts 42. The upper surfaces of the blocks 40 are at angles approximately 5 degrees to the horizontal with the end of the slide adjacent the wicking posts lower than the bar code end of the slide. The angle promotes fluid flow towards the wicking posts 42, which provide the only contact with the slide 1 apart from the block 40. As the wicking posts 42 contact the slide 1 at or near the upper surface of the slide 1, at the lowest end of the slides upper surface, the fluid will tend to wick from the area in the reaction chamber on the slide adjacent the wicking posts 42 and not from other areas, as there are no other wicking points.

It is possible to control the dispenser to dispense fluid onto the slide in various positions. The fluid may be dispensed towards the bard coded end of the slide, or towards the wicking post end of the slide if the covertile is in an open position. It is also possible for the dispenser to dispense in a "staggered waterfall" arrangement where fluid is dispensed in a number of positions up the slide. The covertile may close as the dispenser moves up the slide.

Fluid is dispensed onto the slide 1 in controlled volumes. It has been found that in the current arrangement, fluid does not wick from the reaction chamber 32 down the wicking posts 42 unless one of two conditions are met. Firstly, there needs to be fluid in the reservoir to push fluid through the reaction container. The additional fluid displaces the antecedent fluid, which is removed from the reaction chamber. The antecedent fluid is removed from the reaction chamber via the wicking posts. Thus it is possible to replace a fluid in the reaction chamber by placing fluid in the fluid

reservoir. Secondly, a pump can produce a reduced atmospheric pressure around the wicking posts to cause the pressure differential to draw fluid from the reaction chamber. The reaction chamber may also be drained by reducing air pressure around the wicking posts.

If no new fluid is to be added to the reaction chamber it is possible to drain the reaction chamber by opening the reaction chamber. This is accomplished by sliding the covertile along the slide 1 until the sample is uncovered. The fluid in the reaction chamber will tend to follow the covertile off the sample, draining the fluid via the wicking posts. Alternatively, it is possible to turn on the fan to draw fluid from the reaction chamber, where the covertile can remain in a closed position. A combination of the above is possible.

In some cases, such as where the fluid being applied or in the reaction chamber is particularly viscous, it may be necessary to utilise the pump and apply fluid to the reservoir to cause fluid flow through the reaction chamber. In this way it is possible to change over fluid a controlled way.

The covertile 2 and slide 1 are removed from the apparatus 10 when the reaction is complete and therefore the reaction chamber 32 is unique to each reaction. This eliminates the necessity to thoroughly clean a static reaction chamber as required in other apparatus. Further, the reaction chamber is substantially sealed to the environment reducing evaporation and the possibility of the sample drying out.

As the reaction chamber is formed from a slide and a replaceable covertile, it is relatively inexpensive to form a reaction chamber, and a new, clean reaction chamber is formed for each reaction, reducing cleaning costs and time, as well as eliminating the possibility of cross contamination with previous reactions or cleaning fluids.

The initial fill with the covertile withdrawn (open fill) provides a method of filling the reaction chamber while minimising the formation of voids or bubbles inside the chamber. Due to the reaction chamber having a depth of approximately 100 microns, once the covertile is over the slide forming the reaction chamber, it is difficult to flush the chamber of bubbles or voids. Some of the fluids used in the reactions are

extremely expensive and may be hazardous, and therefore it is desirable to keep their consumption to a minimum.

A suitable initial fill fluid has been found to be a mixture of water and 25% glycerol. Small amounts of glycerol do assist in reducing the incidence of bubble formation, as do larger amounts, however it has been found that in some circumstances 25% glycerol by volume works well. Additives such as detergents (Tween for example) may be included to reduce surface tension, which also have proved beneficial in removing voids in some circumstances.

The use of glycerol reduces the propensity of the fluid to wick from the surface of the slide via extraneous wicking paths. This reduces the number of large voids that form during an initial fill.

To assist in removing any voids that may reside in the reaction chamber after an initial fill, it has been found that a fluid having reduced surface tension and viscosity, but miscible with water, such as an alcohol like isopropanol, is useful as a flushing fluid.

Typically flushing occurs after a heating phase, as increasing the temperature in the reaction chamber can cause bubbles or voids to form. The use of a low viscosity fluid such as isopropanol can assist in moving the bubbles or voids.

Once the reaction chamber is filled with fluid, it is possible to add further fluid without entrapping additional air. Thus, it is possible to change fluids by merely topping up the fluid reservoir, and in some instances, reducing air pressure near the wicking posts. The reaction chamber thus formed exhibits some desirable flow characteristics, in that a new fluid will not tend to mix with the fluid it is replacing. The capillary nature of the reaction chamber does not allow significant turbulent mixing and therefore it is possible to accurately time the changing of fluids without requiring extensive flushing of the chamber or slide surfaces. This allows the start and finish of a reaction to be determined with sufficient accuracy across a range of reactions and fluids.

The speed of the covertile movement and pressure reduction can effect the volume of residual fluids left behind.

In order to promote reactions in the reaction chamber on the sample, it is possible to move the covertile vertically (in the z axis direction as shown in figure 8) on the slide by modulating the load on the clamp 60. The vertical movement assists in mixing the fluid in a vertical direction as well as a direction across the slide (y-axis direction), rather than along its length. Filling and draining the reaction chamber move fluid along the length of the slide (x-axis direction) and this may be assisted by moving the covertile along the x-axis of the slide by moving the arm 44. The blocks 40 may be heated to promote the reaction.

It is desirable in many reactions, for example involving in-situ hybridisation, epitope retrieval, or dewaxing, to heat the fluid in the reaction chamber to a temperature approaching 100 degrees Celsius. In this situation, gas bubbles have been known to form, and the gas bubbles can be difficult to shift. If the bubbles occur on the sample they reduce the amount of fluid exposed to the sample, and can therefore effect the consistency of the result within a sample, as well as between samples on different slides. In such situations it has been found that using covertiles having one or more coatings can reduce the incidence of bubble formation.

Another feature of the reaction apparatus 10 is that the size of the reaction chamber may be varied. Typically the volume of the reaction chamber when the covertile is completely over the slide, termed the closed position, is 150 microlitres. However, if the covertile is not completely closed then the reaction chamber formed between the covertile and slide may be of reduced volume. In figure 8(b) a covertile in a partially closed position is shown, wherein the volume of the reaction chamber would be significantly reduces, for example to 80 microlitres. This example may be useful where samples are small, or placed towards an end of the slid that allows the covertile to form a smaller reaction chamber while still covering the sample. Smaller reaction chambers require smaller volumes of fluids, which is advantageous if the fluids used are expensive or difficult to obtain. The examples of the reaction apparatus allow the position of the covertile to be referenced when dispensing fluid onto the slide. Therefore, when the covertile is in the open position, it is possible to dispense fluid either on top of the tissue sample, or between the tissue sample and the covertile, so

that movement of the covertile to a closed position pushes fluid across the sample while filling the reaction chamber. It is also possible to dispense fluid at a number of positions along the slide, or to dispense fluid on or near the front edge of the covertile.

The following is a description of set up and use of the above-described apparatus.

1. Slide loading: Paraffin-embedded tissue sections (sample 5) mounted onto glass slides are loaded into the slide tray 15 with covertiles 2 and inserted into the receiving zones 14 of the reaction apparatus 10. The user selects desired protocols, run type [ie economy (2/3 of slide) or standard (full slide)] and ensures that the reagents trays 34 containing the necessary reagent containers 39 are loaded into the apparatus 10.

2. Dewaxing: Removal of wax from tissue sections following sectioning is required prior to performing staining procedures. For dewaxing on the instrument the covertile remains in a closed position while dewaxing solution is dispensed by the dispensing means 26 onto the slides, which are pre-heated to 70°C by mounting blocks 40. Slides are incubated for 4 min at 70°C prior to removal of excess dewaxing solution by reduced air pressure around the wicking posts caused by a pump (not shown). Fresh dewaxing solution is dispensed onto the slides for incubation at 70°C for a further 4 min. This process is typically repeated once more for all slides in a rack that require dewaxing. Slides are cooled to ambient temperature and covertiles opened and closed to remove excess dewaxing solution containing residual dissolved wax. All slides are washed with isopropanol applied by the dispensing means one slide at a time, to remove remaining dewaxing solution, and then all slides are rehydrated with distilled water dispensed by the dispensing means.

3. Epitope retrieval: Before IHC and ISH processing can take place, it is necessary to expose epitopes (proteins, DNA, RNA) within the tissue which may have become hidden during the fixation process. On the instrument two protocols may be present:

a. Heat-induced Epitope Retrieval (HIER)

Following dewaxing, all slides receive an initial fill of retrieval buffer (initial fill fluid) (10mM Sodium Citrate/30% Glycerol/0.05% Tween) with the covertile in the open position to facilitate movement of solution down the slide and reduce bubble formation. Covertiles are closed and mounting blocks 40 heat the slides to 100°C for the required retrieval time. After retrieval is finished, slides are cooled by individual flushing with retrieval buffer by the dispensing means.

b. Enzyme-induced Epitope Retrieval (EIER)

Protease solution (ie proteinase K, pepsin, and trypsin) is dispensed onto each slide by the dispensing means and incubated for 10-30 minutes at the desired retrieval temperature (ambient-50°C). After retrieval is complete, each slide is washed with distilled water dispensed by the dispensing means.

4. Immunohisochemistry (IHC): IHC is based on specific binding of antibodies (proteins) to antigens (proteins) in tissue biopsies and specimens. Following the epitope retrieval stage, each slide receives buffer containing Tween-20 from the dispensing means. Each slide is treated with hydrogen peroxide for 5 min at ambient temperature to block endogenous peroxidase activity within the tissue sections and is washed with TWB buffer containing Tween-20, again dispensed by the dispensing means. A primary antibody directed against a specific target protein is applied by the dispensing means to the tissue sample and incubated for 30-60 min. This is followed by a secondary biotin-labelled antibody incubation. Bound antibody is detected by dispensing streptavidin- or alkaline phosphatase-conjugated peroxidase onto each slide, which is visualised by addition of a chromogen (ie DAB, BCIP/NBT), all by dispensed by the dispensing means. Sections are counterstained with hematoxylin, also dispensed by the dispensing means..

5. In situ hybridisation (ISH): ISH allows the detection of specific nucleic acid sequences within a cell. Following the EIER stage, tissue sections are dehydrated by dispensing isopropanol into the reaction chambers of each slide and the covertile moved to the open position to dry the tissue. A fluorescein- or biotin-labelled nucleic

acid probe is applied to the slide and the covertile closed slowly to distribute the probe evenly across the tissue. The probe is allowed to hybridise to its complementary DNA/RNA target in a tissue section for 1.5-2 hours at 37-55°C. Where the target is DNA, the tissue section and probe are first denatured at high temperature (ie 95°C) for 5-10 min prior to hybridisation. Slides are washed by dispensing TWB from the dispensing means using a staggered waterfall rinse to gently remove unbound probe. Following washing, the covertile is moved to the closed position for the remainder of the procedure. Bound probe is detected by applying an anti-fluorescein or anti-biotin antibody conjugated to alkaline phosphatase, dispensed from the dispensing means, which is visualised by addition of an enzyme substrate (BCIP/NBT), also dispensed from the dispensing means.

6. Removal: Once the protocol has been completed for a particular slide tray, the tray may be removed regardless of the status of the other slide trays. As the slide tray may contain slides each having different protocols applied, the tray must remain in the apparatus until all protocols for that particular tray have been completed. An indicator such as a light informs the user when all the protocols to be applied to the slides on the slide tray have been completed.

Once the reaction chamber has been filled it is possible to hold the sample in a buffer for an extended period of time. Fluid in the reaction chamber can be topped up if, for example, some slides reactions are completed but other slides on a slide tray require additional processing. Having three slide trays allows a certain amount of flexibility in that samples that require time intensive processing can be placed in one slide tray, while faster processing may be undertaken on a separate slide tray. An additional slide tray may be entered while one or more slide trays have begun processing, and it is possible to remove a finished slide tray while another slide tray is being processed. The reagent racks 34 may be removed during a process run, if for example, a container empties. Once the reagent rack 34 is replaced, the bar code sensor 33 scans the bar codes on the reagent containers again to ensure that only the correct reagents are applied.

The dispensing mechanism employs a sensor to detect the level of the fluid in the reagent container, and therefore warns the user when the container is running low. This is important as reagent may have a short useful life when not stored properly, and the reagent is also expensive, therefore there are significant advantages in reducing waste.

The sensor may be attached to the pipette to sense when the pipette reaches the surface of the fluid in the reagent container. This allows the volume of a container to be determined, and a warning maybe sent to the operator is fluid levels drop to a predetermined level. The reagent rack may then be removed from the apparatus, the container replaced, whereupon the scanner will determine whether the correct reagent was replaced by reading the bar code on the reagent container. In this way operator error is reduced.

There are a number of variations described herein, but the apparatus is designed to allow a flexible approach to fluid application, reaction time and temperature. It is therefore not intended that the apparatus be limited to particular examples of potential methodology, as variations in fluid application, covertile position and movement.

The protocols that may be applied are varied, and it is possible to apply a different protocol to each sample on a slide in a single rack. Further, it is possible to load a new tray of slides or remove a completed tray of slides while the apparatus is processing another tray of slides.

The claims defining the invention are as follows:

1 A biological reaction apparatus for receiving at least one substrate having a sample located in a sample region, and a separate covertile, such that a reaction chamber is formed between the covertile and substrate over the sample region, wherein the apparatus includes

a locating means to locate the substrate;

a covertile locating means for locating and moving the covertile with respect to the substrate;

a fluid dispensing means for dispensing fluid into the reaction chamber; and

a draining mechanism;

wherein the draining mechanism includes wicking means.

2 The biological reaction apparatus of claim 1 wherein the wicking means include points of contact on the substrate to provide a fluid path to drain fluid from the slide.

3 The biological reaction apparatus of claim 1 or 2 wherein the substrates are supported in the apparatus from underneath.

4 A fill fluid for performing a filling of a reaction chamber, where the fill fluid has a viscosity higher than an antecedent fluid on the substrate.

5 The fill fluid of claim 4 wherein the fill fluid is miscible with water.

6 The fill fluid of claim 4 or 5 wherein the fill fluid has a higher boiling point than water.

7 The fill fluid of claims 4 to 6 wherein the fill fluid leaves no residue on the substrate or sample.

8 The fill fluid of claims 4 to 7wherein the fill fluid is inert to biological reagents and samples.

9 The fill fluid of claims 4 to 8 wherein the fill fluid is a solution comprising glycerol.

10 The fill fluid of claim 9 wherein the fill fluid contains glycerol, water, and buffer.

11 The fill fluid of claims 9 or 10 wherein the fill fluid contains between 2% to 80% glycerol by volume.

12 The fill fluid of claim 9 to 11 wherein the fill fluid contains between 10%-60% glycerol per volume.

13 The fill fluid of claims 9 to 13wherein the fill fluid contains between 20% to 30% glycerol.

14 The fill fluid of claims 4 to 13 wherein the fill fluid includes a surfactant to aid in the disbursement of any bubbles formed within the reaction chamber during a fill cycle.

15 The fill fluid of claim 14 wherein the surfactant is Tween.

16 A receptacle for substrates having receiving means adapted to locate a substrate and a covertile.

17 The receptacle of clam 16 wherein the receiving stations locate and support the substrate, and the covertile is supported on the substrate.

18 The receptacle of claims 16 or 17 wherein the receiving stations support the substrate around part of a periphery of the substrate.

19 The receptacle of claims 16 to 18 wherein the receiving means are defined by a respective aperture having peripheral ledges for supporting the slides.

20 The receptacle of claim 19 wherein the apertures are adapted to receive support platforms from a reaction apparatus, such that when loaded in a reaction apparatus, the platforms support the substrates.

21 The receptacle of claims 16 to 20 wherein the receiving means have a lifting means for lifting the covertiles from the substrate.

22 The receptacle of claim 21 wherein the lifting means are ramps adapted to engage with projections on the covertile.

23 The receptacle of claims 16 or 22 wherein the receiving means have guides allowing the covertile to be moved with respect to the receptacle and slide.

24 A dispenser for a reaction apparatus including a fluid conduit,

a pump connected to the fluid conduit;

a locating means for moving the fluid conduit from a fluid source to a dispensing region.

25 The dispenser of claim 24 wherein the dispenser includes a bar code sensor to detect the type of fluid source and substrate.

26 The dispenser of claims 24 or 25 wherein the dispenser includes a means for determining the volume of fluid remaining in a fluid source.

27 The dispenser of claim 24 wherein the sensor measures a change of capacitance of the fluid conduit to detect insertion into a fluid in the fluid container.

28 A method of dispensing fluid to a slide including the steps of:

loading a reagent receptacle with at least one fluid container;

mounting the reagent receptacle to a reaction apparatus

detecting the reagent receptacle

once the reagent receptacle in detected, initiating a sensor to detect the type of fluid within the at least one fluid container

storing the information on fluid type to allow the fluid to be dispensed onto a slide when required.

29 The method of claim 28 wherein the sensor detects bar codes.

30 A reaction apparatus having a support projection for a slide, a dispensing means and a fluid removal means, where the a support projection is adapted to support a slide from underneath, and a wicking means contacting the periphery of the slide, such that the wicking means provides a wicking path to remove fluid from the upper surface of the slide.

31 The reaction apparatus of claim 30 wherein the support projection is inclined between 0 and 10 degrees to the horizontal providing the mount with a fluid removal region.

32 The reaction apparatus of claim 30 or claim 31 wherein the wicking means is wicking posts.

33 The reaction apparatus of claim 32 wherein the wicking posts are located at the fluid removal region.

34 The reaction apparatus of claims 32 or 33 wherein the wicking means is adapted to extend across a significant proportion of the width of the slide.

35 A reaction apparatus adapted to locate a substrate having a surface containing a sample and covertile having a surface forming a reaction chamber with the sample containing surface, including a covertile engaging means adapted to change the volume of the reaction chamber.

36 The reaction apparatus of claim 35 wherein the covertile engaging means is a clamping mechanism adapted to clamp the covertile to the substrate.

37 A reaction apparatus having a separate substrate tray:

the substrate tray adapted to hold number of slides and covertiles;

at least one receiving station for receiving said substrate tray;

a dispensing means for dispensing fluid onto substrates in the substrate tray

wherein a reaction chamber is formed between the slide and covertile, such that fluid dispensed onto the slides enters the reaction chamber.

38 The reaction apparatus of claim 37 including a number of receiving stations, each station adapted to receive a substrate tray.

39 The reaction apparatus of claim 38 wherein the reaction apparatus has a controller, which allows the fluid to be dispensed onto a substrate on one substrate tray independently of any other substrate tray.

40 A reaction apparatus for receiving a substrate having a sample located in a sample region and a draining mechanism including wicking means for draining fluid from the substrate.

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DATED this 20th day of June, 2002

VISION BIOSYSTEMS LIMITED

By DAVIES COLLISON CAVE
Patent Attorneys for the applicant

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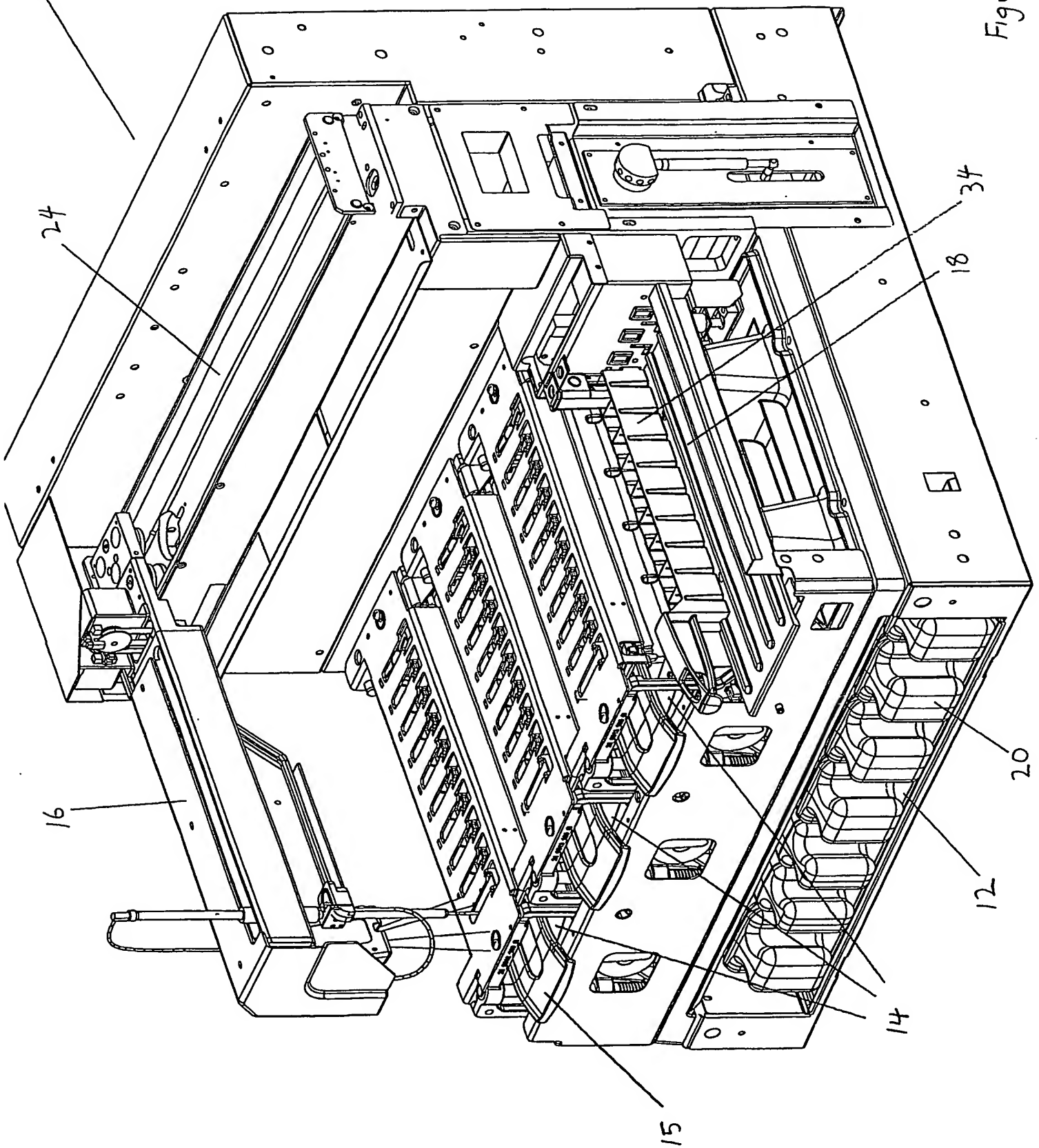

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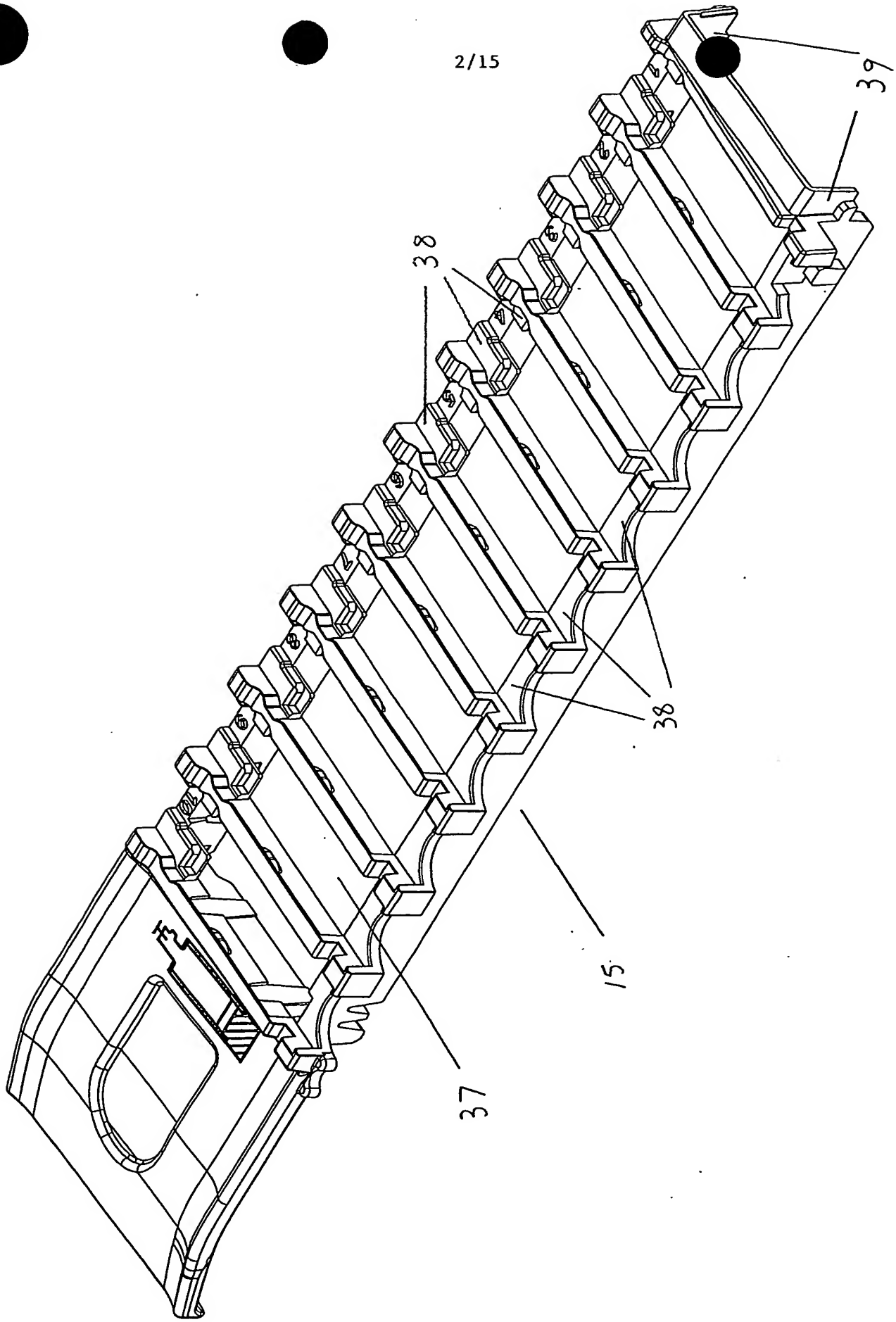

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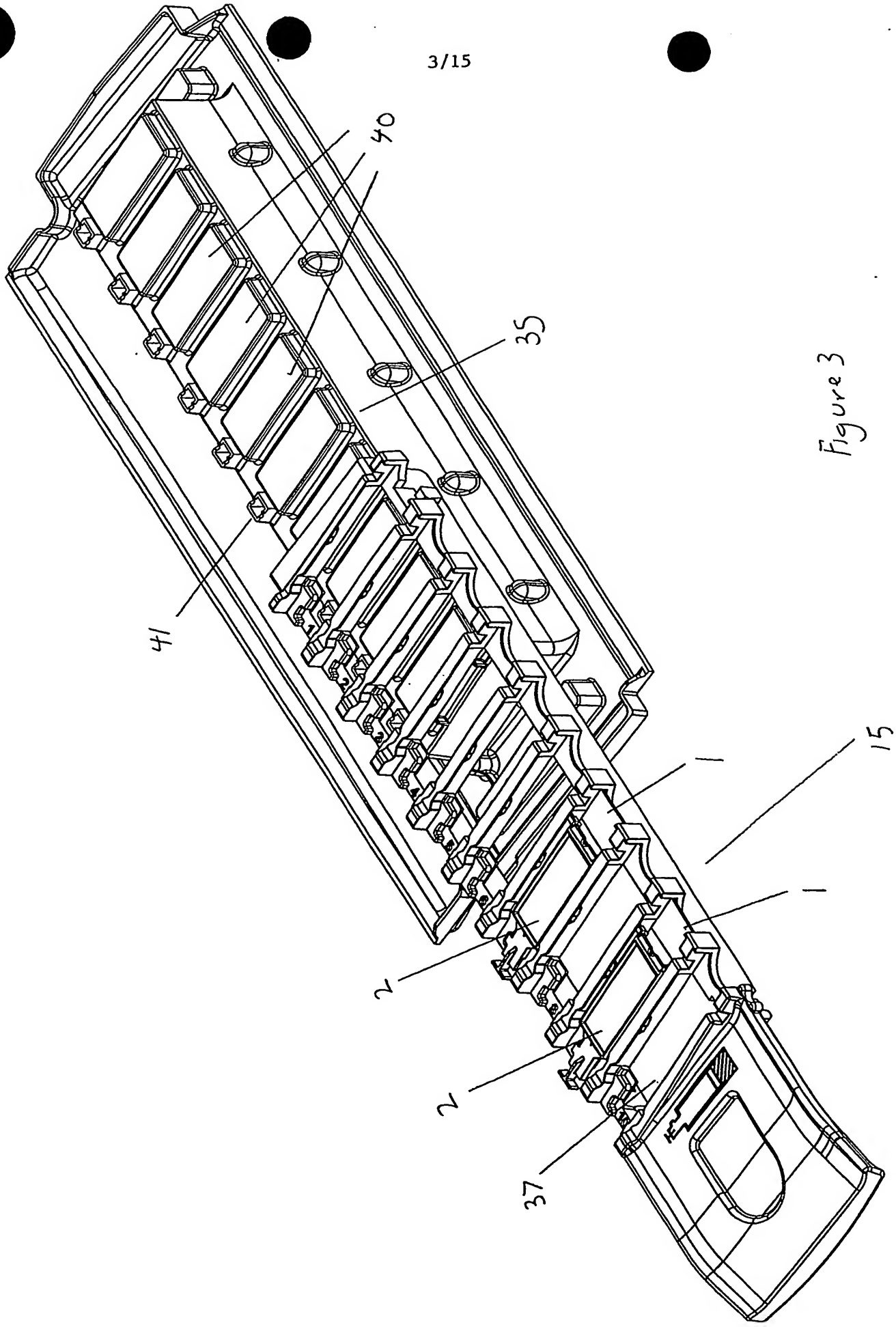

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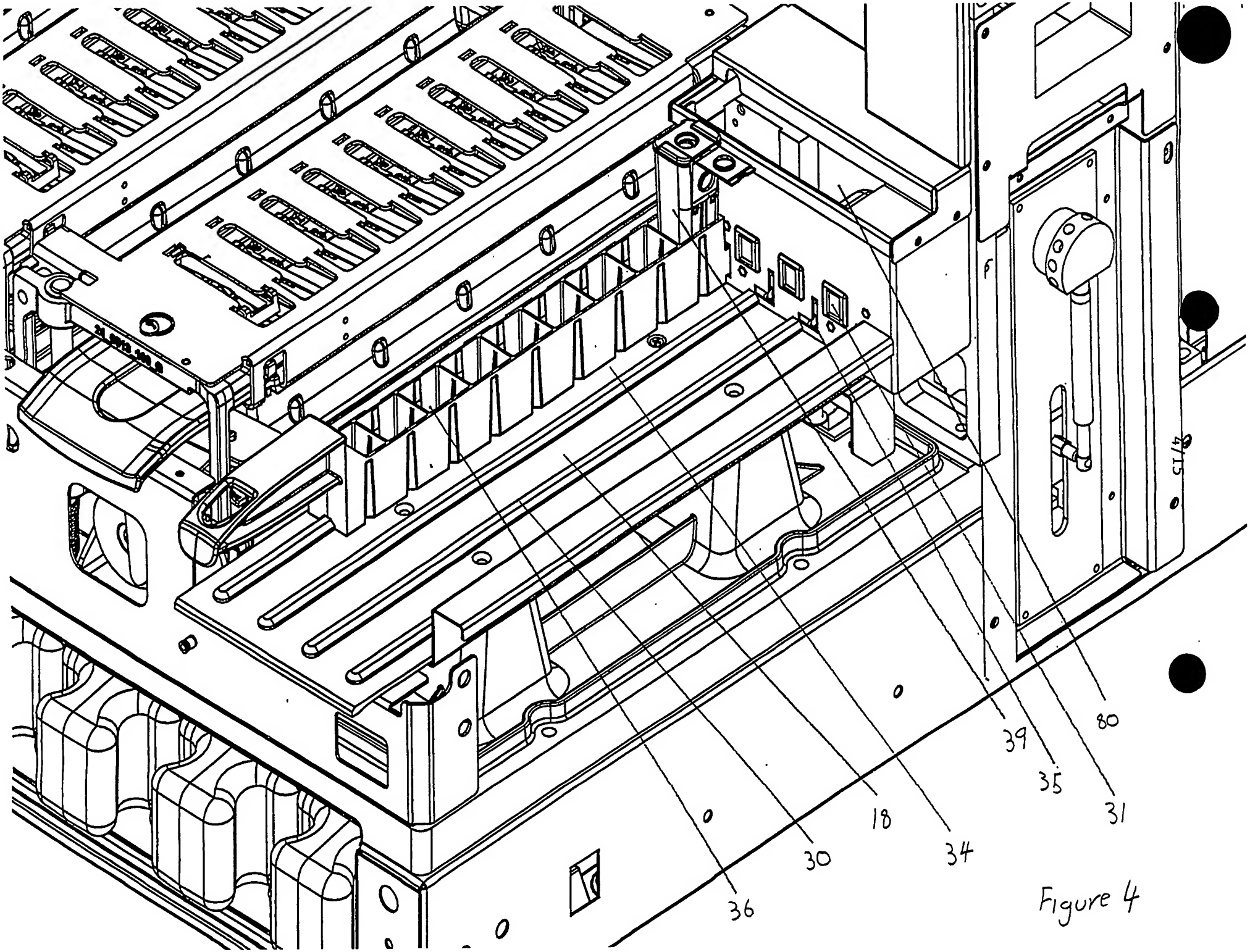

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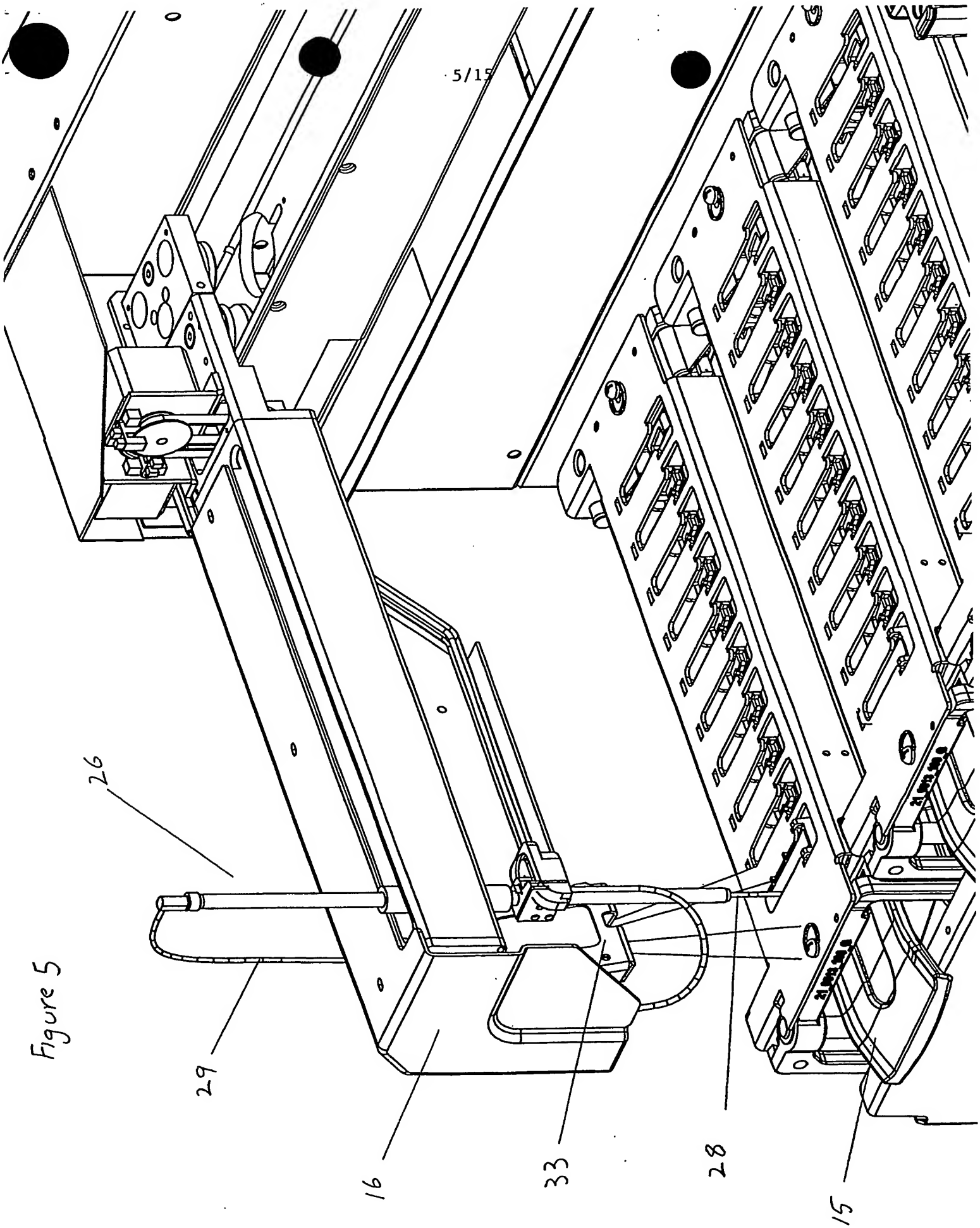
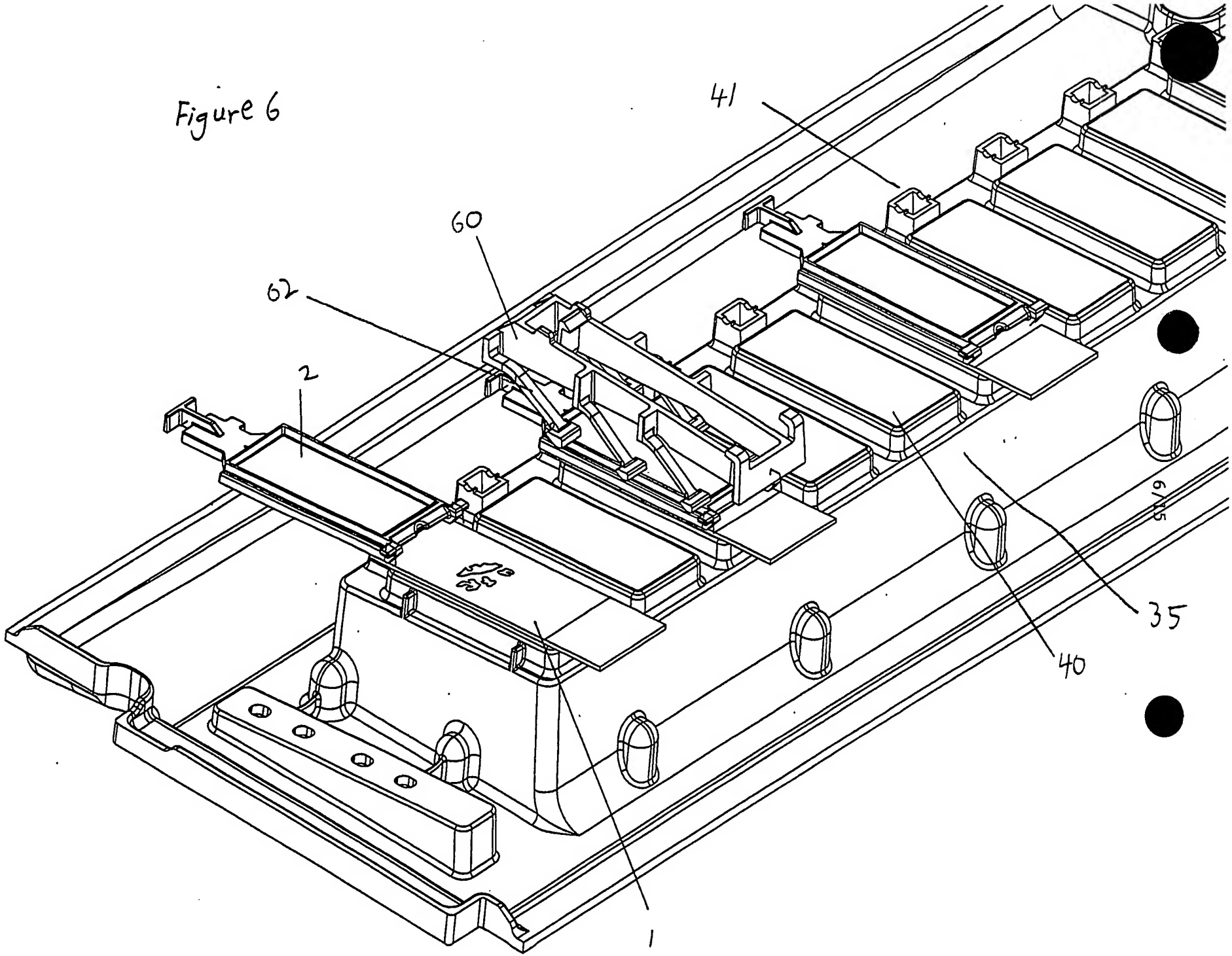
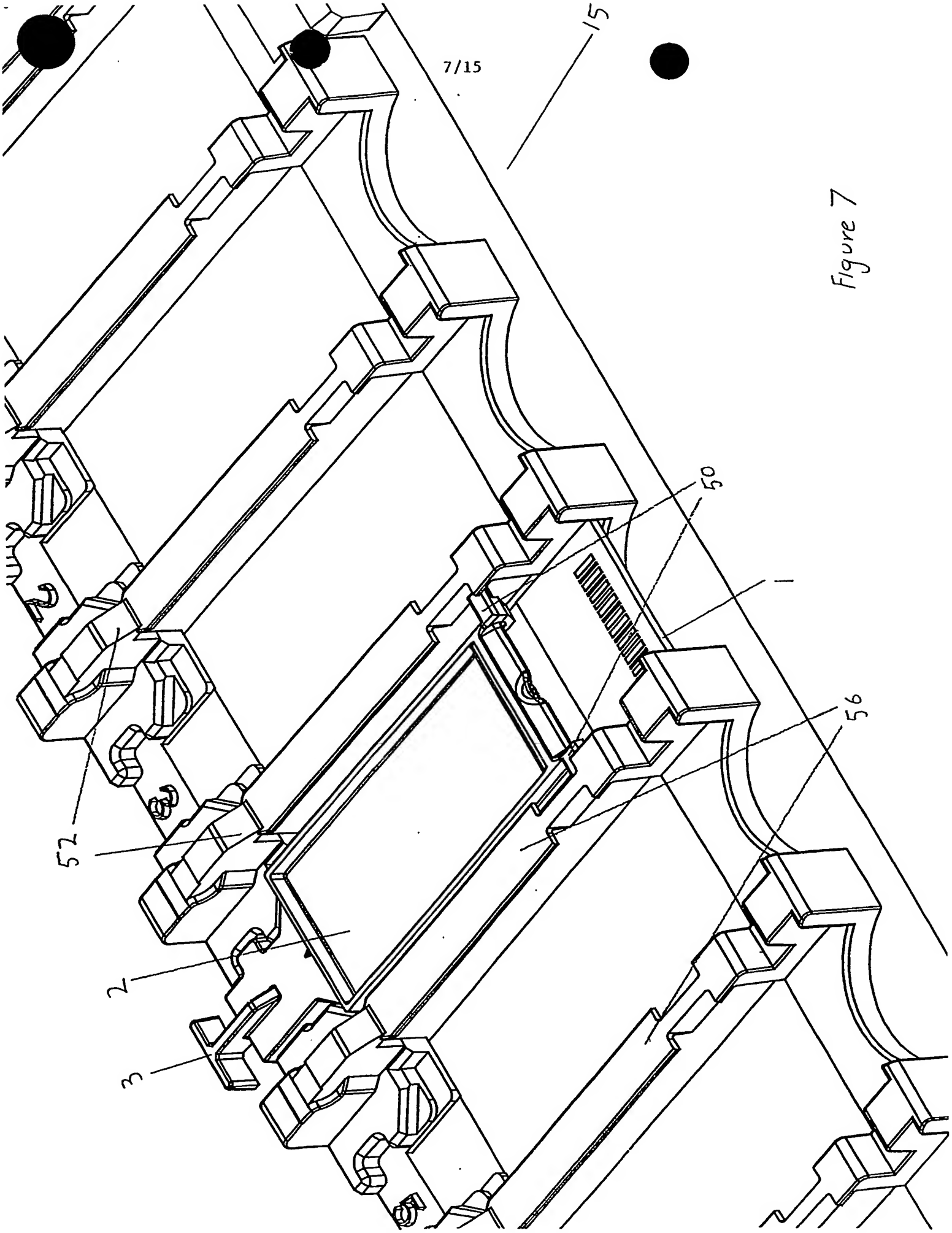


Figure 5

Figure 6





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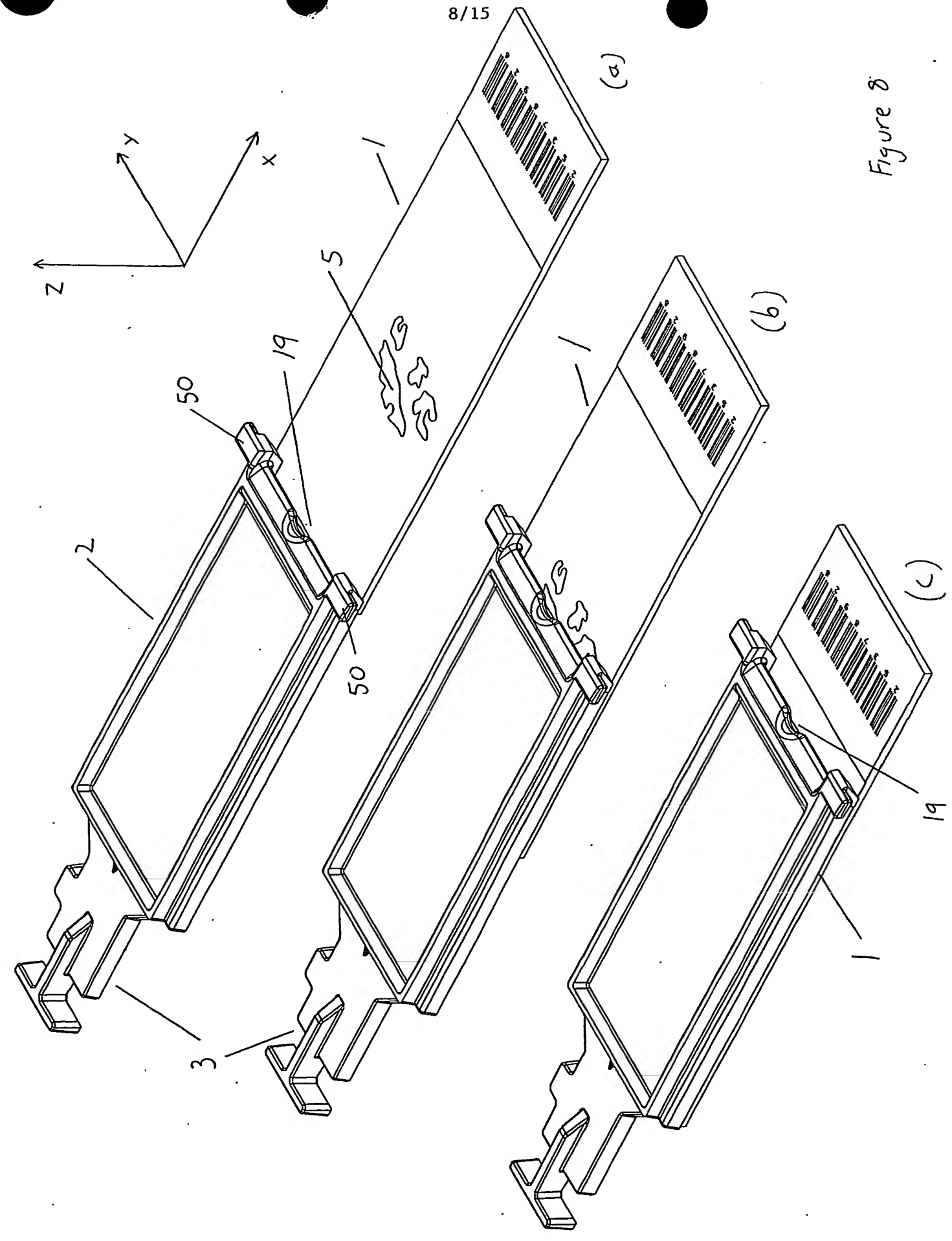

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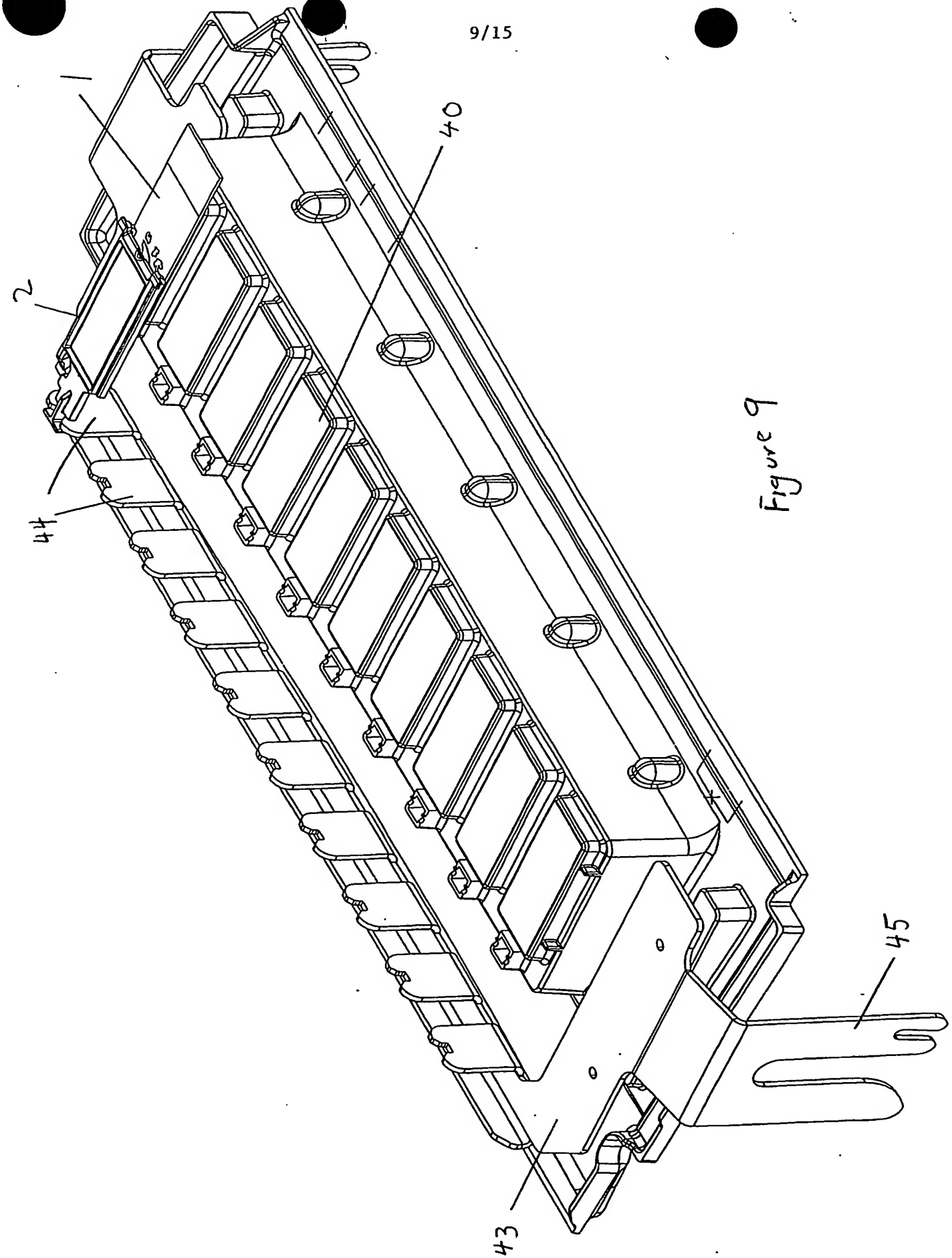

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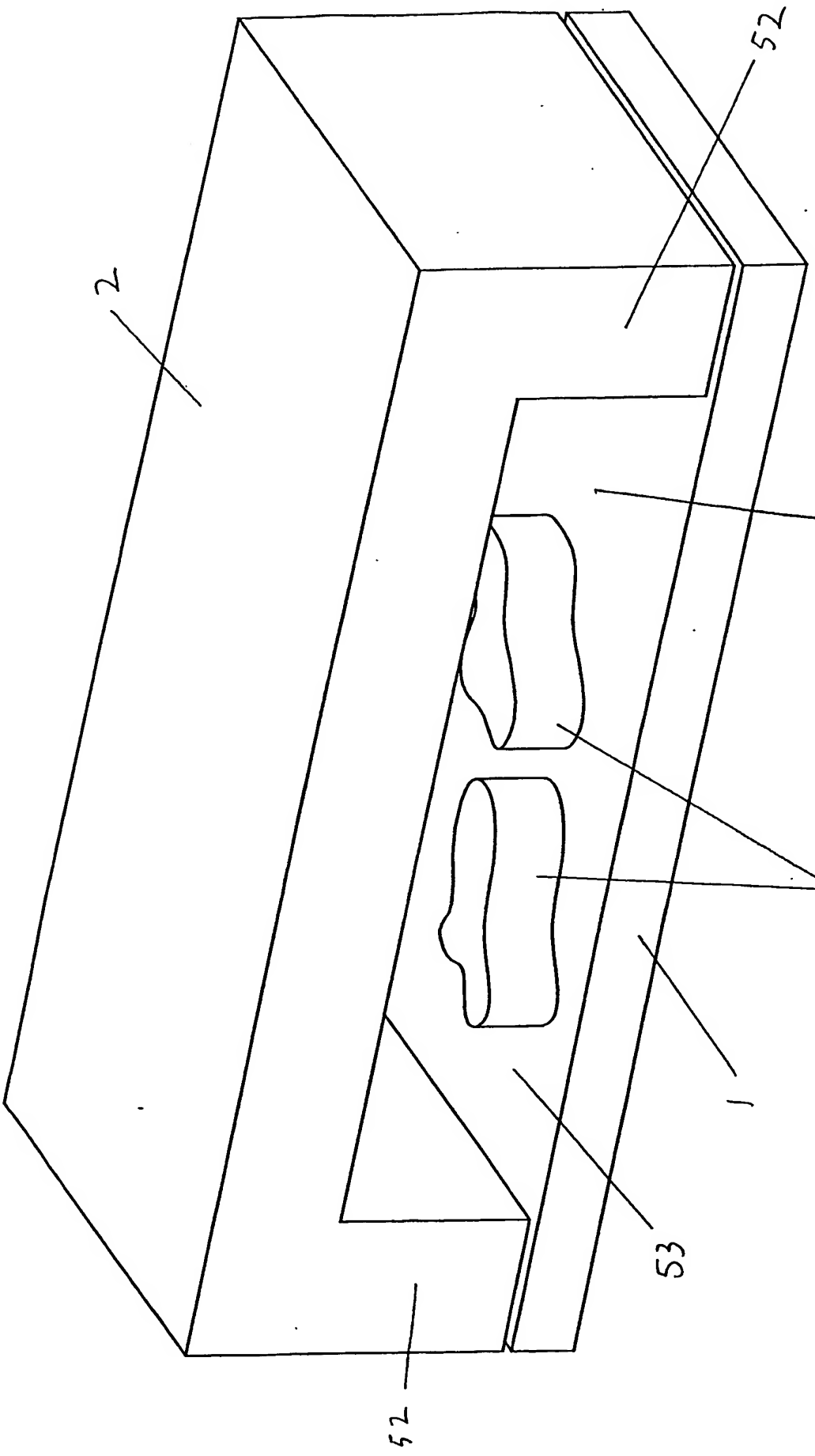

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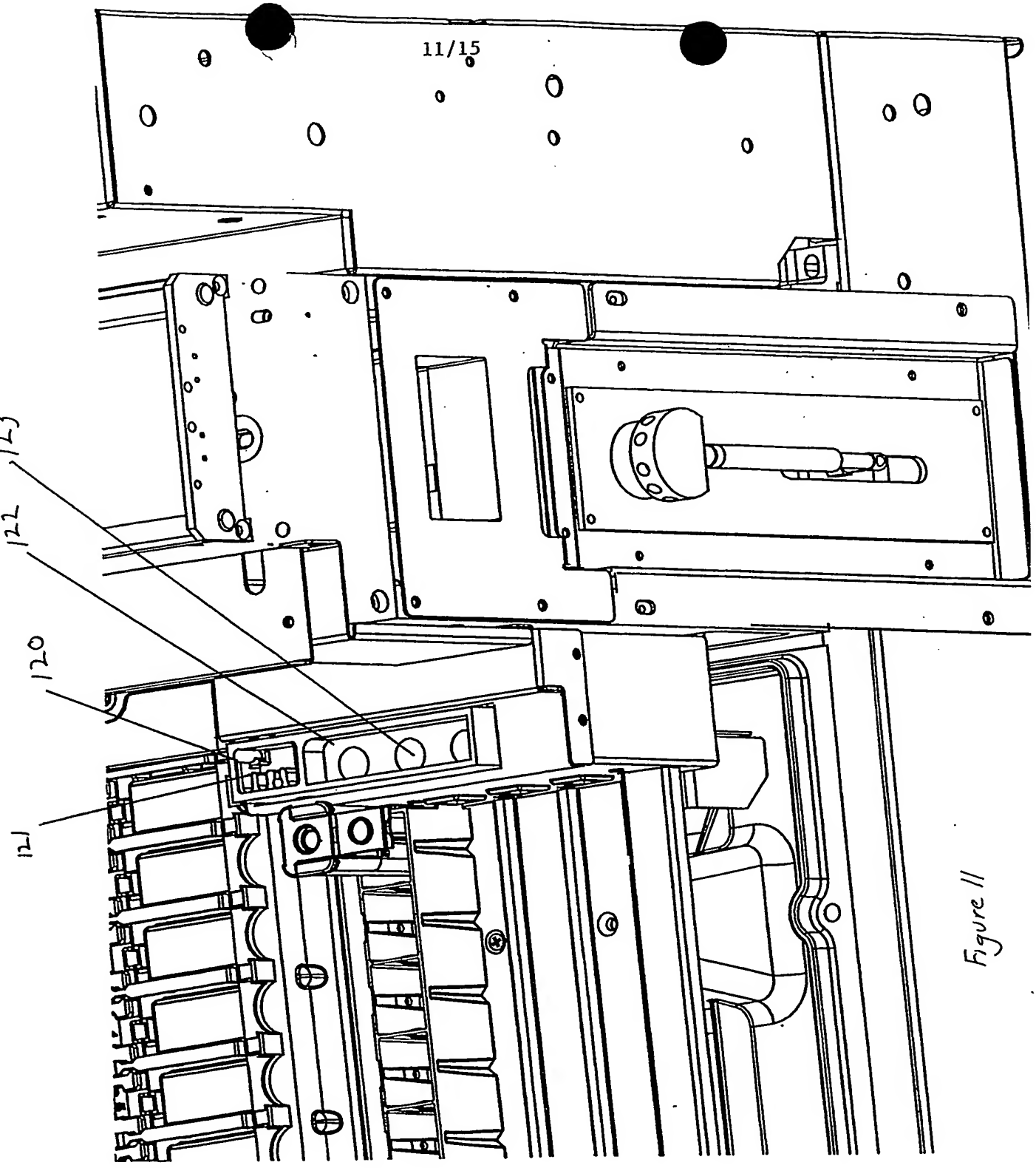

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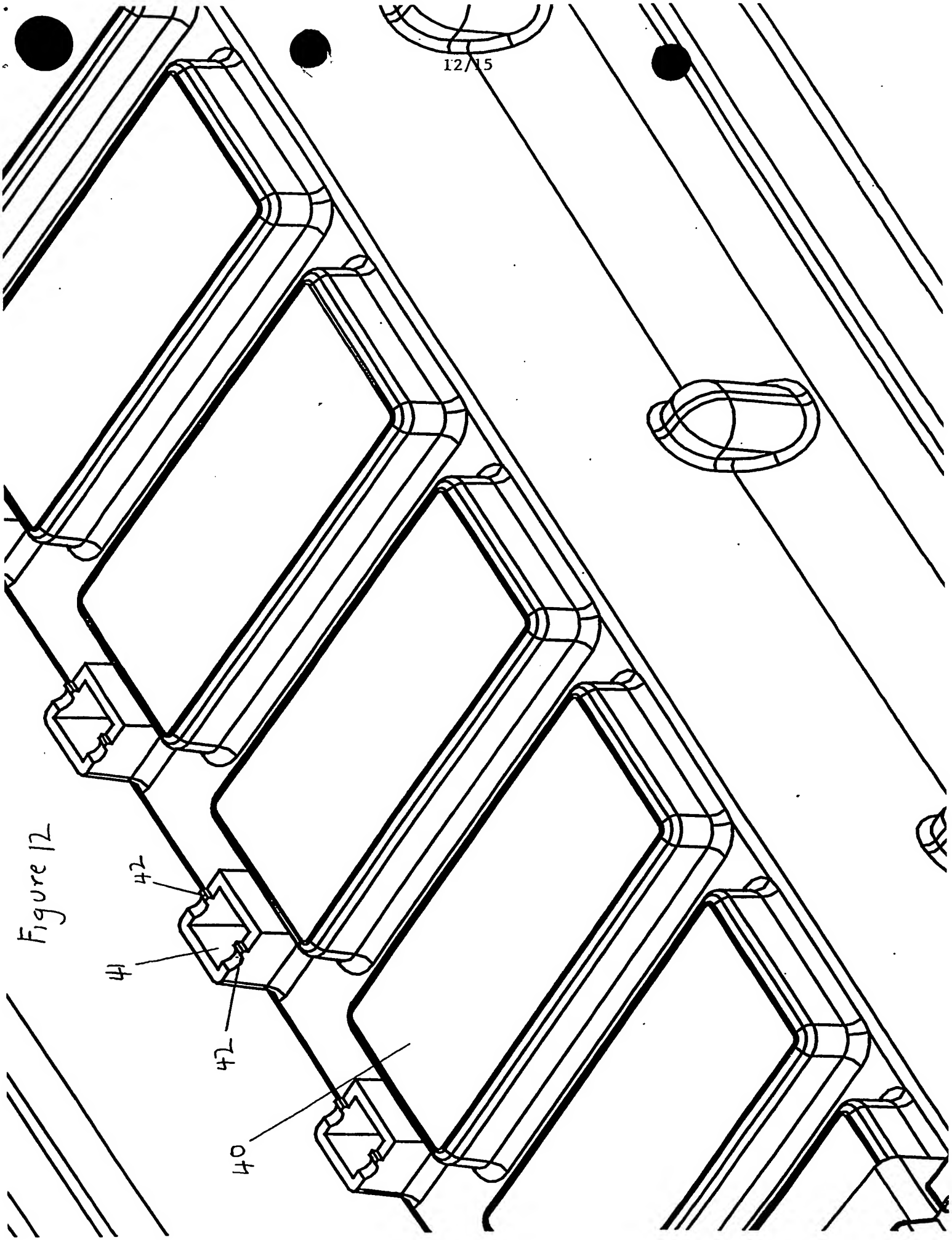

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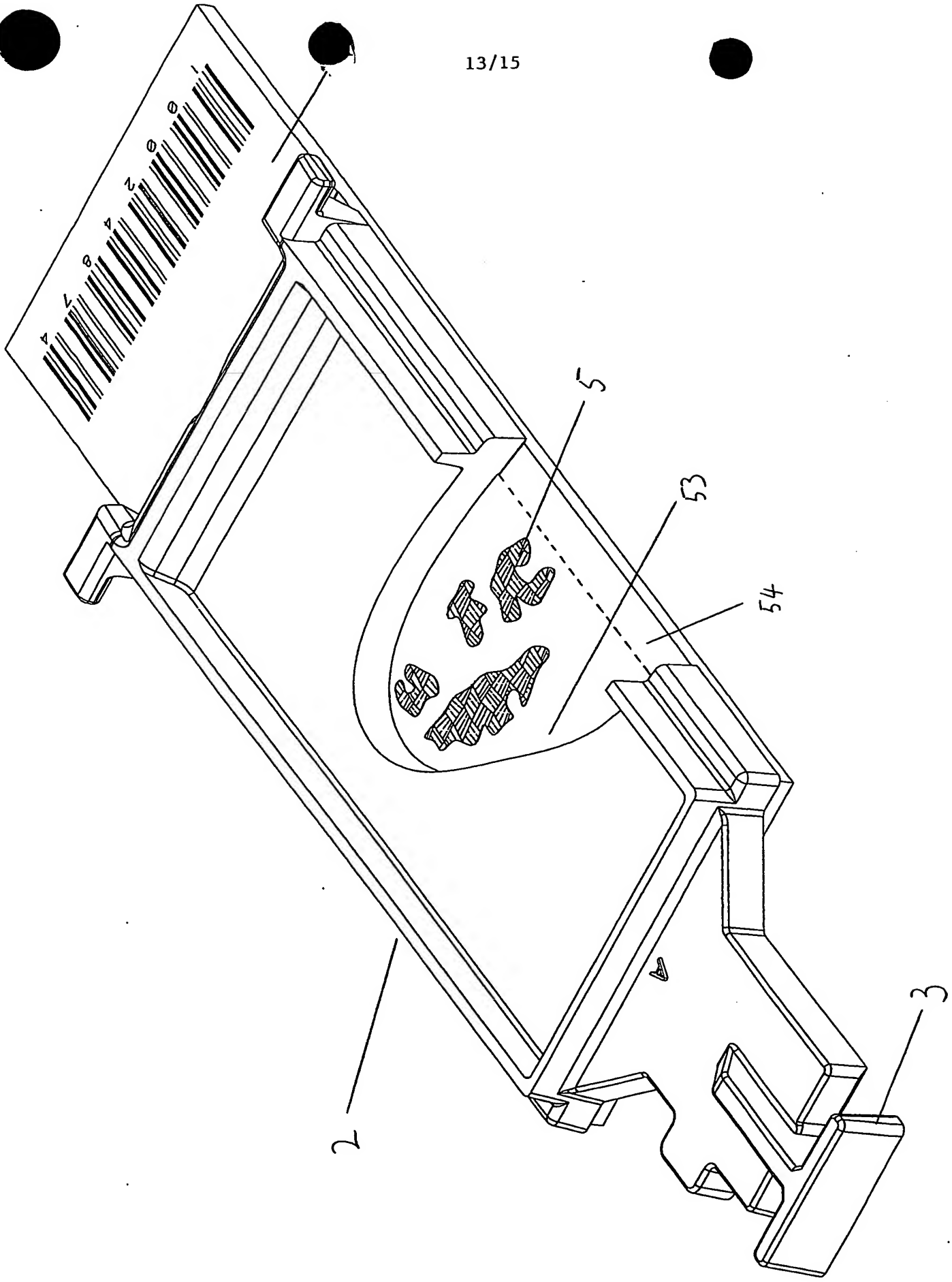

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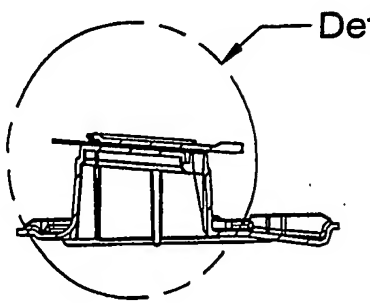
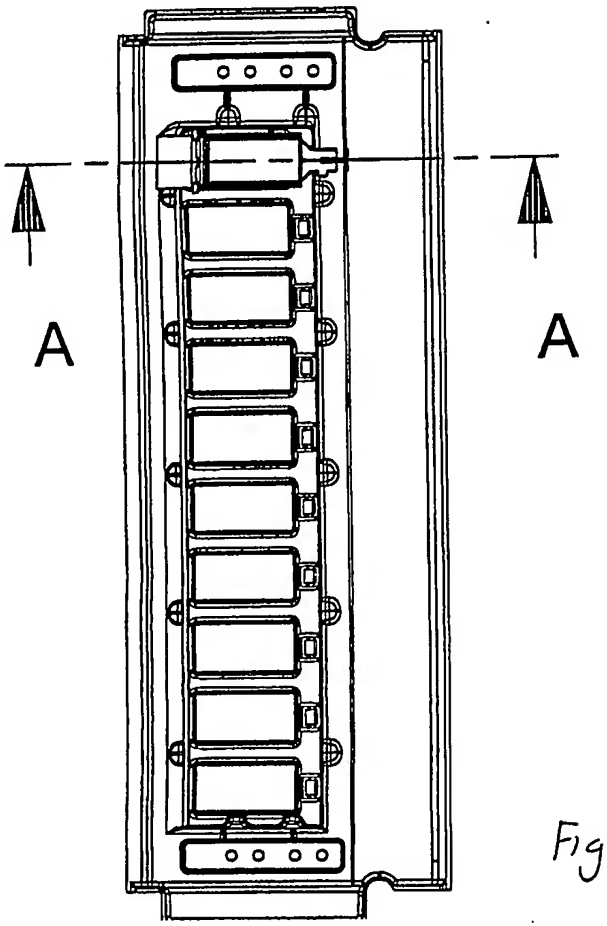


SECTION A-A



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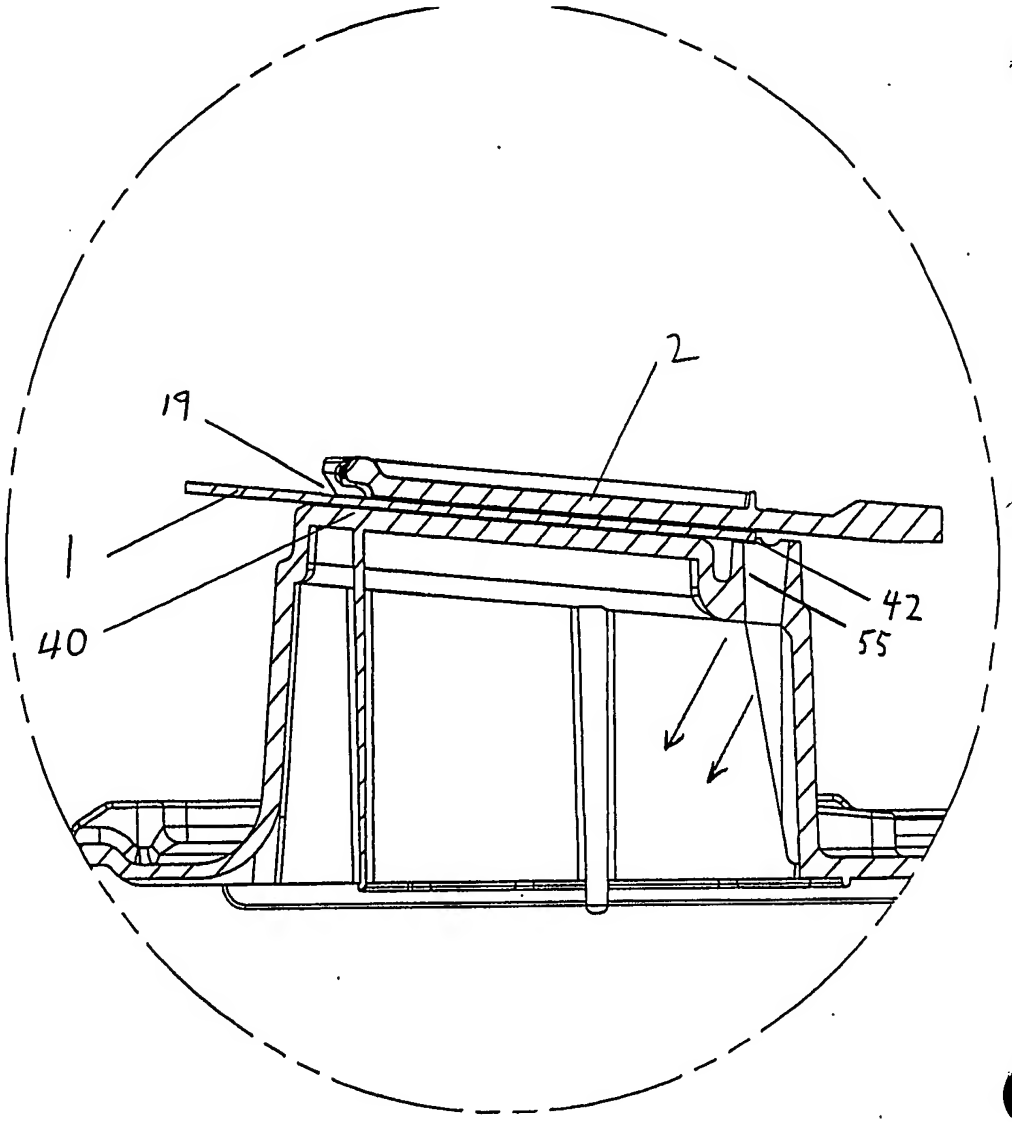

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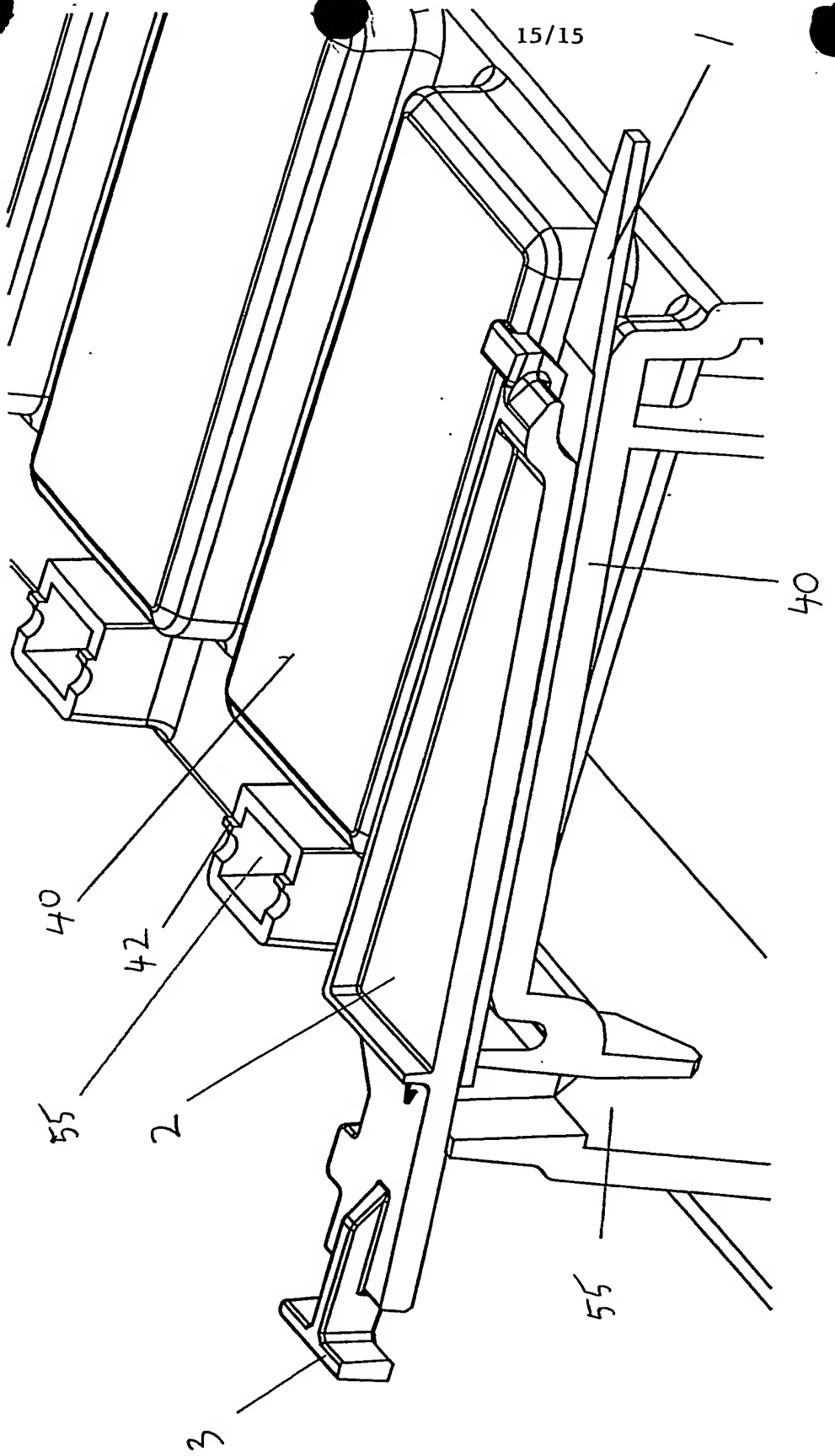

Figure 16